

BIOSYNTHESIS OF SILVER NANOPARTICLES USING ACHYRANTHES ASPERA L STEM EXTRACT

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ABSTRACT

In this study, the synthesis of silver nanoparticles (AGNPs) has been reported capping them through the ethno-botanical species viz., *Achyranthes Aspera* L stem extract. The strong absorption owing to the resonance between the frequency of the electromagnetic field and coherent electron motion from the UV-Vis studies confirmed the presence of NPs that were found to be nearly spherical and ranging in size between 30-80 nm upon characterization with a SEM. The X-ray diffraction analysis established the nature of these NPs to be crystalline and the size of the NPs has been found to be 27 nm. Over a decade now various innovative methods of synthesis of Nanoparticles (NPs) have been in vogue, of which the process involving the aid of an intelligible approach using ethnobotanical species has gained prominence and is today being considered as a large scale substitute to the eco-hazardous production techniques. The process has been identified to be bio-convivial and viable with an ease of escalation for large scale production to cater to the mass supply needs in research and medicine.

KEYWORDS: Biosynthesis, Characterization, UV-Vis, SEM, XRD

INTRODUCTION

The difficulties that humans face to organize themselves in a non-chaotic way has been a complex problem in comparison to and the ease with which nanoparticles in general and silver nanoparticles (AGNPs) are being synthesized cinching them with the use of ethnobotanical species [1-7]. The extracts of these ethnobotanical species are being utilized in the synthesis of silver nanoparticles [8-17]. This method has been found to be paramount to chemical and electrochemical methods, photochemical reactions in reverse micelles [18-21] and other physical methods. These procedures have gained prominence and are being considered as a large scale substitute to the eco-hazardous production techniques due to the achievability of the size, and distribution retaining their morphological structure. Researchers identified that bio-molecules like proteins, phenols and flavonoids not only aid the ions in the nano-size synthesis but are also premeditated in shaping them [22-24]. This procedure has been identified to be bio-convivial and viable with an ease of escalation to the mass supply needs in research and medicine. The nanoparticles possess an unbound appositeness in different territories of biology and medicine, physics and chemistry for applications in catalysis, photonics, biomedicine, antimicrobial activity and optics [25-30]. The uniqueness displayed by these nanoparticles discriminates them from their unique physical and chemical properties of its bulk doppelgänger and their catalytic utilities have fascinated researchers to explore greener pastures of their applicability. A critical review of the literature explicates the precedence of the biosynthesis of nanoparticles (cerium oxide nanoparticles, nickel nanoparticle composite, zinc oxide NPs, crystalline silicon dioxide nanoparticles *etc.,...*). The uniqueness in the properties of AGNPs had resulted in their usufruct in a wide range of applications [31-75].

The ongoing work uses Biosynthesis of silver nanoparticles using the stem extract of *Achyranthes Aspera* L as a capping and reducing agent. After the basic confirmation tests to identify the presence of NPs in the colloid, the sample was further characterized for further analysis.

Taking a cue from characterization results obtained experimentally and upon detailed study of the earlier works a necessity to study the structural properties and establish the necessary theoretical background to evaluate the surface energy of the AGNPs that would be helpful in the explanation of the contraction of their lattice parameters was pursued.

Achyranthes Aspera -Morphology

Achyranthes Aspera L [76-84] is an erect or procumbent, annual or perennial herb of about 1- 2 meter in height, often with a woody base. Stems angular, ribbed, simple or branched from the base, often with tinged purple colour branches terete or absolutely quadrangular, striate, pubescentleaves thick, 3.8 - 6.3 ×22.5 - 4.5 cm, ovate – elliptic or obovate – rounded, finely and softly pubescent on both sides, entire, petiolate, petiole 6 – 20 mm long, flowers are greenish white, numerous in axillary or terminal spikes up to 75 cm long, seeds are sub-cylindrical, they truncate at the apex and are rounded at the base and reddish brown. *Achyranthes Aspera* Lis an important medicinal herb that grows as a weed throughout India. Traditional systems of medicine use all of its parts Wide numbers of phytochemical constituents have been isolated from the plant which possesses activities like antiperiodic, diuretic, purgative, laxative, antiasthmatic, hepatoprotective, anti-allergic and many more medicinal properties. It has been used for a longtime now in the rural parts of India to cure pneumonia, the infusion of the root is used as mild astringent in bowel complaints. For the last few decades or so, extensive research work has been done to prove its biological activities and pharmacology of its extracts. Saponins [85], oleonic acid [93], dihydroxy ketones, alkaloids, long chain compounds and many other chemical constituents have been isolated. The plant has been reported to contain organic compounds like ecdysterone, achyranthine, betaine, pentatriacontane, 6pentatriacontanone, hexatriacontane and tritriacontane[86-93].

MATERIALS AND METHODS

Healthy stem of *Achyranthesaspera* was collected from the surroundings of Khammam, Telangana, India. The stem was long, squarish and cylindrical and thick. It was wild and not fed by the cattle. The extract has been prepared following the procedure of [94-101] as a guideline with a slight modification. Freshly collected stem of *Achyranthesaspera* (50 g wet weight) were cleaned in running tap water, followed by distilled water and then cut into small pieces, crushed with the help of mortar and pestle. The fine colloidal extract was filtered through the fine cotton cloth and the filtrate was collected and diluted with distilled water (300ml). Further, 0.1mM of AgNO_3 solution was prepared and stored in the brown bottle to avoid oxidising. 100ml of the stem extract and 100 ml of AgNO_3 solution were taken in two separate beakers and heated at 60°C for 30 minutes in water bath, cooled and kept for further use.

For synthesis of silver nanoparticles, 95 ml of heated 0.1mM solution of AgNO_3 was added to 5ml of stem extract and stirred with a glass rod for 10 min and the mixture was heated for 30 min at 75°C.using a heating mantle which resulted in a colour changed of the reactants from pale yellow to dark brown. The appearance of brown colour was the first indication of the formation of silver nanoparticles. The solution was then taken off from the heating mantle and cooled. The content was centrifuged at 10000 rpm for 20 minutes and the sediment was collected and stored for further spectral analysis. The sample has later been dried in an incubator and the particles obtained were used for further characterization.

Experimental Procedure


In the single step green synthesis, the stem extract and 1 mM aqueous AgNO_3 solution were added in the ratio of 1:19 and heated up to 75°C for a few minutes until the capping and reduction of AGNPs was complete, which was evident through change in the colour of the colloid. The AGNP solution had been centrifuged (10000 rpm for 15 minutes) and the supernatant was transferred into a dry beaker and stored. The sample had been dried in an incubator and the particles obtained were used for further characterization studies. Characterization results have been recorded using UV-Vis Spectra analysis, XRD measurement, SEM analysis of silver nanoparticles.

RESULTS AND DISCUSSIONS

Initially the change in the colour of the solution and later through the characterization studies of UV-Vis, SEM and XRD analysis substantiated the bio-reduction of aqueous silver ions to silver nanoparticles.

UV –Visible Spectra Analysis

The nanoparticles were preliminarily characterized by UV-Visible Spectroscopy to analyse the nanoparticles. As the stem extract has been mixed with the aqueous solution of the Silver ion complex the colour changed to brown due to the excitation of the surface plasma vibrations which indicated the formation of the Silver nanoparticles[102]. UV-Visible Spectrograph has been recorded as a function of time by using quartz cuvette with distilled water as the reference. The reaction has been carried at 90°C and the colour change has been observed at different time intervals of 30, 30, 90 min. Figure 1 shows the variation of absorbance after the completion of the reaction respective reaction times through the curves a, b, and c at 90°C temperature. The UV spectrum absorption has been recorded at 432nm which was a confirmation of the formation of the AGNPs and the broadening of the peak indicated that the particles are poly-dispersed [103-105]. The much heavier ionic core of these particles induce polarization of the electrons from an incoming wave of the electric field[105]that would result in a restoring force which creates an in phase dipolar oscillation.

And the origin of the observed colour has been due to a strong absorption owing to the resonance between the frequency of the EM field and coherent electron motion. The difference in the energies of the conduction and valency bands in the case of AG is very low permitting free movement of electrons thus, leading to the oscillation of free electrons of AGNPs in resonance with the light wave ultimately resulting in SPR [105, 107-109].Furthermore, UV-Vis is a paramount tool of analysis of the metastable solution of single particles [109-111].Beyond this metastable state the dominant Van der Waal's forces sequel the cluster formation [111-112]. The flattened spectrum indicated the saturated state of the reaction and reduction of AGNPs for this concentration of the stem extract [113-114]. The framework of Mie scattering theory to analyze optical spectra has been applied for the determination of the particle size of the AGNPs present in the stable suspension [102-118]. The *full width at half maximum*(FWHM) of the peak  has been obtained from the spectral graph Figure 1 as follows.

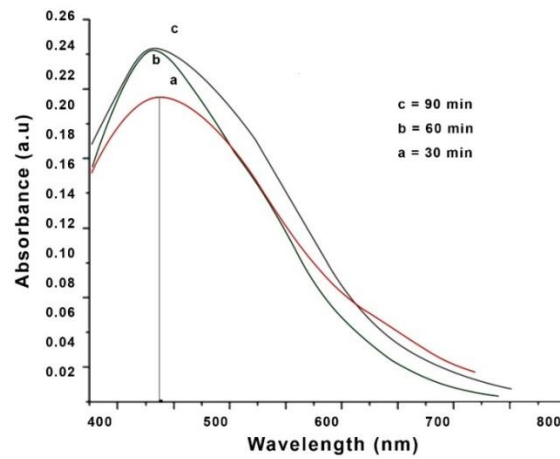


Figure 1: UV-Visible Absorption Spectrum of Achyranthes Aspera Stem Extract, Silver Nanoparticles

$$\omega = \frac{(\epsilon_0 + 2n^2)c}{2N_c} \frac{m u_F}{e^2 D}$$

where, the frequency independent part of complex form of the particle are, ϵ_0 – dielectric constant (.49), n – refractive index(0.15016), c - velocity of light, m - mass of the electron, u_F - electron velocity at the Fermi Energy, N_c - Number of electrons per unit volume, e - charge of the electron, and D - Diameter of the particle. The UV-vis spectra are fitted using log-normal function

$$P(D)dD = \frac{1}{\sqrt{2\pi}\sigma D} e^{\left(-\frac{\ln^2\left(\frac{D}{D_0}\right)}{2\sigma^2}\right)}$$

to obtain the standard deviation (σ) as the system of the poly dispersed nanoparticles obeys log-normal size distribution function. Using σ and the mean particle size obtained particle size distribution curve has been generated as in Figure 2. The particle diameter has been determined. It was found to be ~ 31 nm.

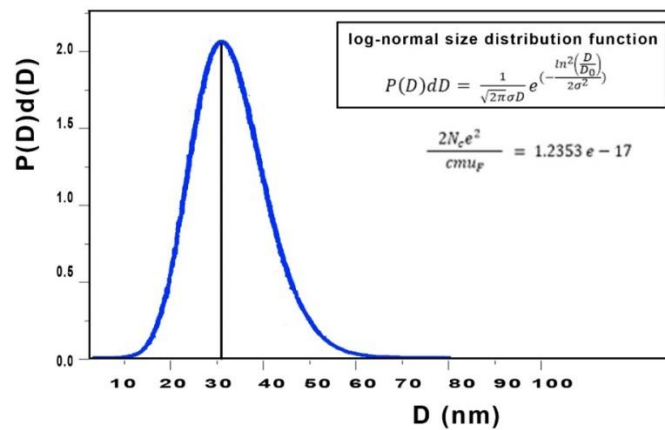


Figure 2: The Log-Normal Distribution

SEM Analysis

The Ag Nanoparticle pellet obtained after centrifugation has been re-dispersed in deionized water several times

before morphology was characterized. The AGNPs formed were preponderantly spherical with uniform shape Figure 3. The SEM image exhibits the formation of porous surface with spherical nanoparticles [119-126] that were clearly distinguishable in size ranging between 30- 80nm.

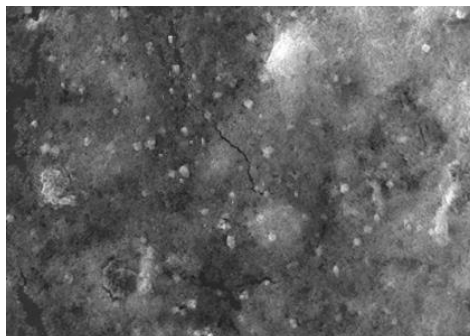


Figure 3: SEM Image of Silver Nanoparticles Formed from *Achyranthes Aspera* Stem Extract

XRD Analysis

X-ray diffraction has been a convenient method for determining the mean size of single-crystal nanoparticles. After repeated centrifugation followed by re-dispersion of the pellet of silver nanoparticles into 10 ml of sterile distilled water and freeze drying the purified silver nanoparticles, the structure of the synthesized silver nanoparticles were investigated with an XRD (RIGAKU-D Machine). The sample was casted on a glass plate and the analysis was made at the voltage of 40 kV and current of 40 mA. The source used was copper K α line. Based on the XRD result, the crystalline domain size was calculated from the width of XRD peaks using Scherrer's equation [127] which relates the size of sub-micrometre particles, or crystallites, in a solid to the broadening of a peak in a diffraction pattern.

$$D = \frac{K \lambda}{\beta \cos \theta} \quad \text{Where } \beta = \frac{\pi}{180 * FWHM}, \quad \lambda = 1.540598 \text{ \AA}, K\lambda = 0.94 * 1.540598 \text{ \AA} = 1.4482$$

The XRD shows that silver nanoparticles formed are crystalline [127-135]. Comparing the Spectral distribution with the standard powder diffraction card of Joint Committee on Powder Diffraction Standards (JCPDS), silver file No. 04-0783. The XRD study confirms / indicates that the resultant particles are (FCC) Silver Nanoparticles. [128]. The Experimental diffraction angle [2 θ] and Standard diffraction angle [2 θ] of the Table 1 are in agreement [129]. Strong Bragg reflections that correspond to (111), (200), (220) and (311) reflections of silver metal with face centred cubic symmetry have been identified. The high intense peak has been observed at (1 1 1) reflection. The intensity of peaks reflected the high degree of crystallinity of the silver nanoparticles.

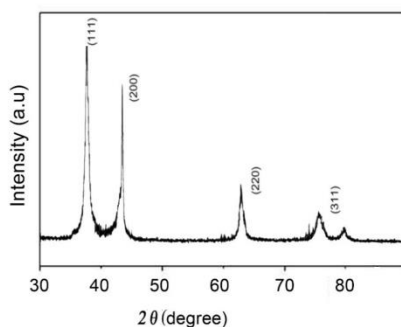


Figure 4: XRD Showing Peak Indices (111, 200, 220, 311) & 2 θ Positions

Table 1

hkl	Value of 2θ In Degree	FWHM in Degrees	FWHM in Radians	Value of D in nm
111	37.875	3.15	0.0548	27 nm
200	44.625	3.2	0.0556	28 nm
220	63.125	2.0	0.0348	48nm
311	75.375	1.9	0.0331	55.2nm

CONCLUSIONS

Biosynthesis of AGNPs has been reported using the stem extract of the ethnobotanical species *Achyranthes Aspera* as a reducing and capping agent.

The UV-Vis studies attributed the change in the colour of the colloid to the strong absorption owing to the Surface Plasmon Resonance between the frequency of the electromagnetic field and coherent electron motion. The results revealed the absorption peak at 432 nm due to the electron oscillations that collectively gathered around the surface of silver particles. The particle size obtained using the framework of Mie scattering theory has been found to be about ~31 nm.

Clearly distinguishable spherical AGNPs with sizes ranging between 30- 80nm have been proclaimed by the SEM images. The optical and electronic properties of the AGNPs being largely shape dependent, the synthesized AGNPs in the current method being predominantly spherical in shape would condign the applications where uniformity of these properties would be a basic requirement.

The XRD studies have divulged the synthesized NPs to be crystalline in nature with the size of the particles calculated using the Scherer's equation yielding a value of ~27 nm.

Finally, this procedure has been found to be consummate for the synthesis of AGNPS and the results are being further analyzed and being interpreted to further study the variation of the lattice parameters with size, shape and morphology as an annex to accomplish control on these parameters, an objective of the subsequent work.

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